IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

GEERLINGS, Maurits W.

Control Number: 90/005,214 Group Art Unit: 1641

Filed: December 21, 1998 Examiner: B. Nguyen

RE: U.S. Patent 5,246,691

For: RADIOIMMUNOTHERAPY USING ALPHA-PARTICLES EMISSION

DECLARATION UNDER 37 CFR 1.132

Assistant Commissioner of Patents
Washington, D.C. 20231

April 24, 2000

Sir:

I, David A. Scheinberg, M.D., PhD, have the position of Professor, Member and Chief, Leukemia Service, as well as Head, Hematopoietic Cancer ImmunoChemistry Laboratory, Memorial Sloan-Kettering Cancer Center in New York City, New York;

I had begun investigating the use of alpha emitters for therapy by 1990, first focusing on bismuth-212 as an alpha emitter for conjugation. Subsequently, on the urging of Dr. Geerlings, we shifted focus from bismuth-212 to bismuth-213 and actinium-225 as sources of alpha radiation for therapy with immunoconjugates.

In addition to conducting human trials with leukemia using bismuth-213 conjugated with antibodies having specificity for leukemia cell associated antigens, we also began *in-vitro* studies using actinium-225 as the radiometal.

We assessed cell killing activity *in-vitro* using actinium-225 conjugated to HuM195 as the targeting moiety.

For the in-vitro trials we targeted HL60(CD33 positive cells) deposited in quantities of 50,000 cells per well in 96 well plates.

The cells were exposed to varying concentrations of actinium-225 conjugated to HuM195 for 48 hours. As a control, the CD33 negative RAJI cell line was also exposed to [Ac-225] HuM195.

After 48 hours, [H-3]thymidine was added to each well, and the cells were incubated for 4 hours and thereafter harvested by paper filtration. [H-3]thymidine uptake indicated the presence of viable cells. The ratio of [H-3]thymidine taken up by cells exposed to [Ac-225] HuM195 versus the [H-3]thymidine taken up by cells grown in media, which were not exposed to the actinium conjugate, provided the percentage determination of viable cells per dose level.

The attached graph illustrates the efficacy of actinium-225 conjugates targeted specifically to epitopes characteristic of a particular cell line. Two different doses of alpha radiation were applied to the cells, 0.12 Ci/g and 0.0012 Ci/g. The results indicate that with the higher level of radiation (0.12 Ci/g) the conjugate of actinium-225 bound to HuM195, having specificity for HL60, results in the essentially complete destruction of viable cells. The results also illustrate that even at the lower level of radiation (0.0012 Ci/g) the immunoconjugate having specificity for HL60 cells showed a greater reduction in viability than even 100 fold higher levels of radiation (0.12 Ci/g) administered using the same immunoconjugates to RAJI cells that have no epitopes to which HuM195 will bind. These results illustrate the principle of using actinium-225 in an immunoconjugate having specificity for target cells.

These in-vitro studies compare favorably with the results we obtained using bismuth-213 bound to HuM195 as illustrated in Figure 5, page 172 of Nikula et al. "Alpha Emitting Bismuth Cyclohexylbenzyl DTPA Constructs of Recombinant Humanized Anti-CD33 Antibodies: Pharmacokinetics, Bioactivity, Toxicity and Chemistry," The Journal of Nuclear Medicine, Vol. 40, No. 1, January 1999, a copy of which is attached hereto. Targeting 20,000 HL60 cells per well, [Bi-213] HuM195 reached cell viability of less than 10% at concentrations providing between 10^{-6} and 10^{-5} Ci/ml. The graph submitted herewith shows that [Ac-225] HuM195 reached the same levels at less than 10^{-9} Ci/ml, based on the present assay using 50,000 cells per well.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present patent.

4/26/00

David A. Scheinberg, M.D., PhD

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Assistant Commissioner of Patents
Washington, D.C. 20231

April 4, 2000

Sir:

I, David A. Scheinberg, M.D., PhD, have the position of Professor, Member and Chief, Leukemia Service, as well as Head, Hematopoietic Cancer ImmunoChemistry Laboratory, Memorial Sloan-Kettering Cancer Center in New York City, New York;

I, am co-author with Matte Strand and Otto Gansow of the publication entitled "Targeting In Erythroleukemia Mice: Radioiodinated and Chelated Radiometal-Conjugated Monoclonal Antibody" Monoclonal Antibodies Drug Development, John Jacob Able Symposium for Drug Development, 1982, pages 159 - 171;

As mentioned in that publication, we had begun by 1980 to consider using alpha emitters for therapy, and for alpha emitters we and other investigators in the field focused on bismuth-212 with virtually all the efforts going forward with respect to bismuth-212;

It was only after the urging of Dr. Geerlings that we and, later, after we presented our data showing utility, others in the field

diverted our focus from bismuth-212 to actinium-225 and bismuth-213 as sources of alpha radiation for therapy.

Dr. Gearlings made me aware that the physical characteristics of the emissions of Bi-213/Ac-225 made these isotopes the most likely isotopes that could be feasibly used clinically for alpha-particle based radioimmunotherapy. After several years of investigation this concept has now been validated in humans.

Since involvement with Dr. Gearlings began we have now conducted alpha therapy trials in humans with leukemia, with safety and anti-leukemia activity demonstrated. We have also achieved success *in-vitro* using actinium-225 as the radiometal that suggests that similar activity of this latter isotope may be possible in humans as well.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present patent.

David M. Scheinberg, M.D., PhD